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ISOZYME EVIDENCE AND PHENETIC RELATIONSHIPS AMONG SPECIES IN *ASTER* SECTION *BIOTIA* (ASTERACEAE)

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ABSTRACT

Aster section *Biotia* is an eastern North American group of asters having a base chromosome number of $x = 9$. Phenetic analyses of isozyme gene frequency data show that the species native to the eastern deciduous forest region, *Aster divaricatus* ($2n = 18$), *A. chlorolepis* ($2n = 36$), *A. schreberi* ($2n = 54$), and *A. macrophyllus* ($2n = 72$) are very closely related and perhaps form an increasing polyploid series. Isozyme data reveal that the two species whose ranges are restricted to the Piedmont, *A. mirabilis* ($2n = 18$) and *A. jonesiae* ($2n = 54$), are very closely allied and are more distantly related to the previous group. The endemic and midwestern *A. furcatus* ($2n = 18$) is isozymically most unlike the other species in the section. Isozyme data do not rule out the possibility that the hexaploids, *A. schreberi* and *A. jonesiae*, originated by retrogressive polyploidy rather than by progressive (increasing) polyploidy. The hypothesis that *A. chlorolepis* arose from *A. divaricatus* by autopolyploidy is supported by the electrophoretic data. The relationships of the diploids to one another and the mode of origin of *A. macrophyllus* are not clarified by the isozyme analyses.

Key Words: *Aster* section *Biotia*, isozymes, electrophoresis, phenetics, eastern North America

INTRODUCTION

The North American species of *Aster* form a large, taxonomically difficult and evolutionarily complex assemblage (Allen, 1984, 1986; Dean and Chambers, 1983; Gray, 1880, 1882, 1884; Jones,

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1980a, 1980b; Semple and Brouillet, 1980a, 1980b; Shinners, 1941; Torrey and Gray, 1841). Taxonomic difficulties in the genus mostly involve species delimitations (Allen, 1984, 1986; Jones, 1980b, 1984), but even in cases where species are well defined, relationships between them are not. Hybridization and introgression (Avers, 1953; Wiegand, 1928, 1933), polyploidy (Dean and Chambers, 1983), and phenotypic plasticity (Jones, 1978a, 1978b) have been cited as the primary contributors both to blurring of species boundaries and to uncertainty about phylogenetic relationships.

From all other *Aster* groups, the eastern North American *Aster* L. section *Biotia* DC. ex Torrey and A. Gray can be distinguished by the following combination of characteristics: long-petiolate basal and lower cauline leaves with cordate-based, toothed blades; upper cauline leaves with toothed margins; mature cypselas that are fusiform with 7 or more prominent ribs; heads with the outermost phyllaries densely ciliolate and obtuse to rounded at the apex; capitulescences corymbiform; and base chromosome number $x = 9$. Judging by morphology, the most closely related groups are subgenus *Aster* subsection *Spectabiles* A. Gray and subgenus *Doellingeria* (Nees) A. Gray from eastern North America, and subgenus *Aster* section *Radulini* (Rydberg) A.G. Jones from western North America (Jones, 1980a; Jones and Young, 1983).

Aster section *Biotia* comprises three diploids: *A. divaricatus* L., *A. furcatus* Burgess in Britton and Brown, and *A. mirabilis* Torrey and A. Gray; one tetraploid: *A. chlorolepis* Burgess in Small; two hexaploids: *A. jonesiae* Lamboy and *A. schreberi* Nees; and one octoploid: *A. macrophyllus* L. Three of these species, *A. divaricatus, A. schreberi,* and *A. macrophyllus,* are common in New England (Lamboy, 1990, Ph.D. dissertation, University of Illinois, Urbana; Seymour, 1969), and *A.* ×*herveyi* A. Gray, a hybrid between *A. macrophyllus* and *A. spectabilis* Aiton, is endemic to New England and New York.

The seven members of *Aster* section *Biotia* and the one hybrid may be distinguished by means of the following taxonomic key.

KEY TO *ASTER* SECTION *BIOTIA* AND ONE SYMPATRIC HYBRID

1. Plants with stipitate-glandular hairs on the phyllaries and peduncles 2

2. Blades of lower cauline leaves broadly ovate or ovate, cordate at the base; innermost phyllaries usually less than 7 mm long, never reflexed, squarrose, or twisted at the apex; rhizomes long stoloniform; in deciduous or coniferous forest areas, eastern North America . . . . . *A. macrophyllus*

2. Blades of lower cauline leaves ovate to narrowly ovate, rounded to cordate at the base; innermost phyllaries always more than 7 mm long, at least some of them reflexed, squarrose, or twisted at the apex; rhizomes caudiciform or short to long stoloniform; usually in sandy soil of coniferous forest areas, New England . . . . . *A. ×herveyi*

1. Plants without stipitate-glandular hairs on the phyllaries and peduncles 3

3. At least some of the phyllaries squarrose 4

4. At least some of the phyllaries obovate or oblanceolate; ray florets usually 16 or more, white or lavender; disk florets 50 or more; phyllaries usually more than 50; North and South Carolina . . . . . *A. mirabilis*

4. Phyllaries ovate, elliptic, or lanceolate; ray florets usually 15 or fewer, blue or violet; disk florets usually 25 or fewer; phyllaries fewer than 50; Georgia and Alabama . . . . . *A. jonesiae*

3. None of the phyllaries squarrose 5

5. Leaves scabrous above, rough below; primary and secondary veins particularly prominent below; disk florets yellow at anthesis, turning greenish with age; on substrates of or derived from limestone or sandstone; Iowa, Missouri, Illinois, Wisconsin, Michigan, and Indiana . . . . . *A. furcatus*

5. Leaves glabrous to scabrous above, glabrous below; primary and secondary veins not particularly prominent below; disk florets yellow at anthesis, turning magenta with age; on various substrates; United States east of the Mississippi River, southern Ontario, and southern Quebec 6

6. Blades of lower cauline leaves broadly ovate to ovate, cordate with broad sinuses at the base, usually with more than 30 teeth along each

side of the leaf; plants often forming large clonal patches of mostly sterile basal rosettes; southern Ontario, and northeastern United States, scattered localities in the Appalachian Mountains and Midwest ..... *A. schreberi*

6. Blades of lower cauline leaves ovate, cordate at the base with sinuses lacking or very narrow, usually with fewer than 30 teeth along each side of the leaf; never forming large patches of mostly sterile basal rosettes 7

7. Longest peduncles usually longer than 2.0 cm; heads wider than 2.5 cm when the rays are fully extended; involucre 0.5–1.0 cm tall; high mountains in Virginia, Tennessee, North Carolina, and Georgia ... . *A. chlorolepis*

7. Longest peduncles usually shorter than 2.0 cm; heads narrower than 3.0 cm when the rays are fully extended; involucre 0.35–0.75 cm tall; at lower altitudes throughout eastern North America .. *A. divaricatus*

Possible interspecific relationships, which have been postulated on the basis of morphology, geography, and chromosome number (Lamboy, 1990, op. cit.), are shown in Figure 1. The only phylogenetic statement that can be made with a reasonable degree of certainty, though, is that the montane tetraploid, *Aster chlorolepis,* arose from *A. divaricatus* by autotetraploidy (Lamboy, 1991, at review). The former species is, in many morphological characters, simply a larger version of the latter. In addition, *A. chlorolepis* is endemic to the higher altitudes within the range of the *A. divaricatus.* Both of these pieces of evidence support the autotetraploid origin of *A. chlorolepis.*

The relationships between the other taxa are not nearly as clear. In fact, there are at least two plausible origins for most of the species. For example, because it is found in the northern two-thirds of the range of *Aster divaricatus,* and because it shares inflorescence size and shape characters with *A. divaricatus,* it is possible that the hexaploid, *A. schreberi,* arose from *A. divaricatus* by means of autopolyploidy and subsequent backcrossing to the diploid.

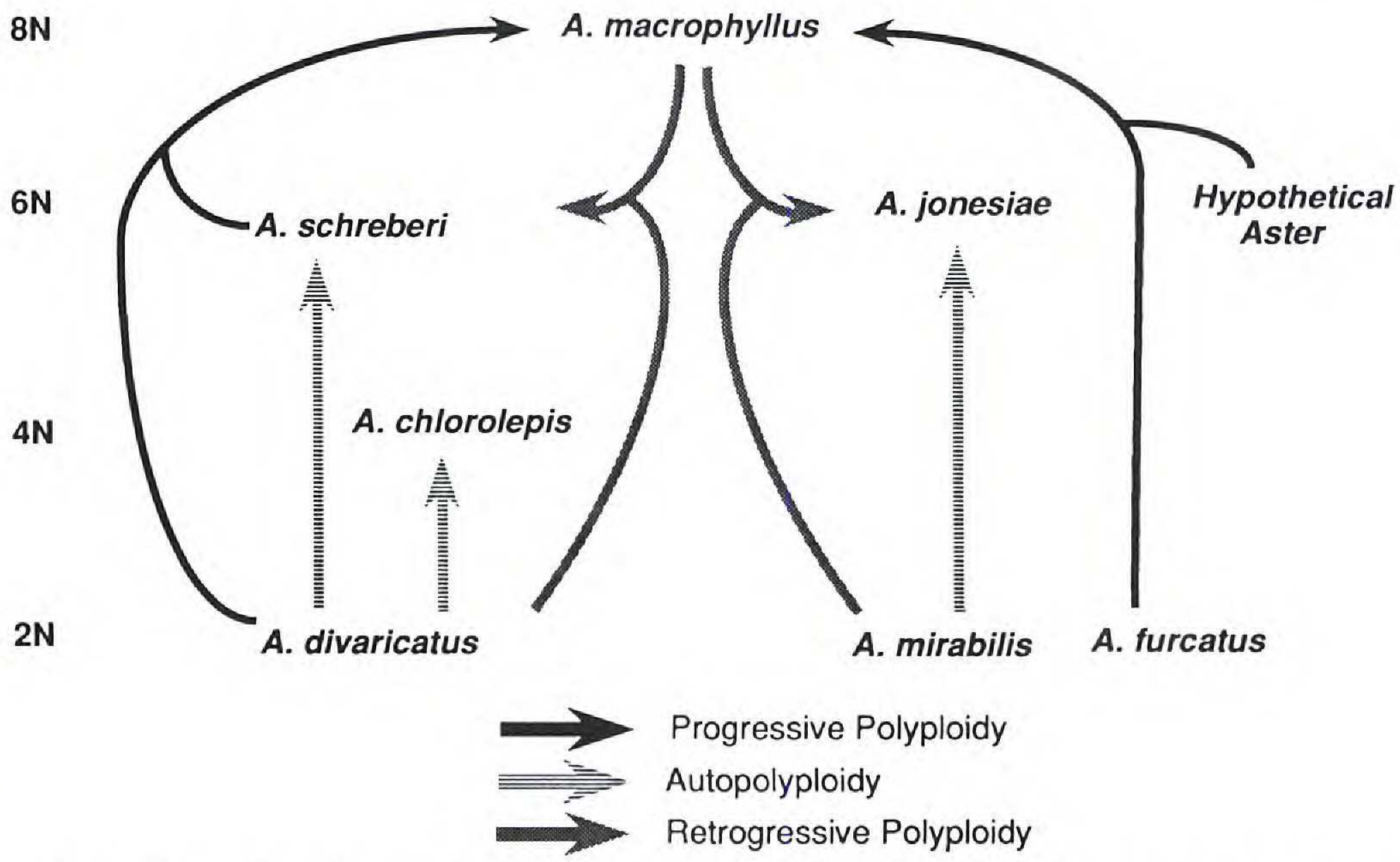

Figure 1. Possible ancestor - descendant relationships between species in *Aster* section *Biotia.* Arrows point from ancestors to descendants. Polyploid levels of the species are indicated at the left. Note that for some species, e.g., *Aster schreberi,* the information currently available does not strongly favor one mode of origin over the others.

On the other hand, plants of *Aster schreberi* are similar in size to *A. macrophyllus,* and they exhibit the same leaf shape, leaf toothing, and clonal colony formation as well. This similarity suggests that *A. schreberi* may have arisen by retrogressive polyploidy from a hybrid between *A. divaricatus* and *A. macrophyllus.* Retrogressive polyploidy as defined by Lamboy and Jones (1988) is: "the formation of a hybrid derivative from a successful cross between two species, one a high polyploid and the other a lower polyploid or diploid, resulting in a sexually reproducing species having a ploidy level intermediate between that of the two parents."

The other hexaploid in *Aster* section *Biotia, A. jonesiae,* has squarrose phyllaries and a caudiciform rhizome system, characteristics it shares with the diploid, *A. mirabilis,* but it has stem diameter, overall plant size, and basal and lower cauline leaves much like those of the octoploid, *A. macrophyllus.* Both *A. jonesiae* and *A. mirabilis* are native to the Piedmont of the southeastern United States, and *A. macrophyllus* grows close by in cool shaded habitats in the southern Appalachians. It is possible, therefore, that *A. jonesiae* arose either from *A. mirabilis* by autopolyploidy

with backcrossing to the diploid or by retrogressive polyploidy from putative parents *A. mirabilis* and *A. macrophyllus.*

Morphology provides little information about the relationships of the diploids to one another. Geographic and ecological evidence suggests, though, that *Aster divaricatus* may be the oldest of the three diploids. This species is native to the eastern deciduous forest and possesses the most extensive range of the three species. In contrast, both *A. furcatus* and *A. mirabilis* have restricted ranges and occupy less stable habitats. The former is found in rocky places along watercourses, and the latter is native to the transition zone between southern Appalachian hardwoods and oak-pine forests. Before *A. divaricatus* can be accepted as the oldest of the diploids, however, additional evidence must be obtained.

Finally, the octoploid, *Aster macrophyllus,* may represent the endpoint of the increasing polyploid series: *A. divaricatus* to *A. chlorolepis* to *A. schreberi* to *A. macrophyllus,* since plant height, stem diameter, and cauline leaf size form an increasing series in this order. On the other hand, *A. macrophyllus* could be the allopolyploid derivative of a hybrid (e.g., between *A. furcatus* and some other *Aster* species), for *A. furcatus* and *A. macrophyllus* have been found historically in a number of the same locations in the midwest, and they both possess scabrous leaves, large capitula, and tall, thick stems.

Because morphology does not clarify the origins of and the relationships among species in *Aster* section *Biotia,* we conducted an electrophoretic analysis using isozymes of all members of the section to shed more light on their phenetic relationships. In addition to providing an answer to the fundamental question of what the genetic distances are between the taxa, analysis of electrophoretic data can help answer other questions of taxonomic interest, such as whether gene frequency data support the origin of *A. chlorolepis* from *A. divaricatus* by autopolyploidy. Such data may also throw light on three questions: did the two hexaploids, *A. jonesiae* and *A. schreberi,* originate by retrogressive polyploidy or via the more usual progressive polyploidy; from what parentage did *A. macrophyllus* arise; and how are the diploids related to one another?

The goals of the electrophoretic analysis of *Aster* isozymes were:

1. To obtain genetic distances between species and to construct phenograms from this information.

2. To determine whether genetic distances between putatively different species have numerical values that are usually found for different species, or whether they are in the range usually found for different populations of the same species.
3. To use unique alleles, if any, present in the diploids to assess ancestry of the polyploids, and to determine if they are likely to be auto- or allo-polyploids.
4. To examine genetic distances in order to find species that are particularly closely related, which may be evidence that they have diverged from one another relatively recently.

No published information is available on isozymes in *Aster.* Gottlieb (1981) analyzed seven taxa, two of which he called *Aster,* and five species of *Machaeranthera.* However, one species, *A. riparius,* has since been transferred to *Machaeranthera,* and the remaining species, *A. hydrophilus,* should probably also be transferred to *Machaeranthera,* or at the very least, should be taken out of *Aster.* The only previous numerical work on the phylogeny of members of the genus *Aster* was done by Jones and Young (1983), and their study, using morphological characters and chromosome numbers, assessed the phylogeny of North American supraspecific groups in the genus but did not examine phylogenies of species within any section.

MATERIALS AND METHODS

Living plants were obtained from populations in the eastern United States. Since the purpose of this study was to examine interspecific rather than intraspecific genetic relationships, and since the members of *Aster* section *Biotia* are native to most of the eastern United States, emphasis was placed on collecting specimens from as many localities throughout the entire geographic range of each species as possible, rather than on collecting many specimens from a single population. Results obtained by previous workers show that this procedure should give a reasonably accurate representation of the genetic variation in *Aster* species which are outcrossing (Jones, 1974; Lamboy, unpubl.), even though population information will not be available (Avise, 1975; Crawford, 1983).

Living plants were greenhouse-grown in Urbana, Illinois, and leaf material was collected on the day of grinding. Leaves were cut near the base of the petiole with a razor blade, transferred to

plastic sandwich bags, and then placed on ice in a plastic cooler. All leaf material was ground within two hours of collection, although tests showed that material left overnight at 4°C before grinding showed no loss of enzyme activity. Two grams of leaf material, cut with scissors, were placed in a mortar with 5–8 times (by volume) liquid nitrogen, and ground by hand with a pestle. Before the powdered tissue was allowed to thaw, it was transferred to a test tube containing grinding buffer at 4°C consisting of: .029 M sodium tetraborate, .017 M sodium metabisulfite, .100 M sodium ascorbate, .016 M diethyldithiocarbamic acid, and .100 M potassium dihydrogen phosphate adjusted to pH 7.5. Five percent (w/v) PVP-40 and 1.0% (v/v) 2-mercaptoethanol were added to the buffer before use. The mixture was homogenized at maximum speed with a Polytron homogenizer, and the homogenate was transferred to plastic centrifuge tubes and centrifuged at 10,000 rpm for 10 minutes at 0°C. The supernatant was poured into microfuge tubes and stored at −100°C.

The extracts were thawed on ice and the liquid absorbed onto filter paper wicks. Enzyme separation was performed on 14% horizontal starch gels as described by Shaw and Prasad (1970). The following enzyme systems were used: triose phosphate isomerase (TPI) [E.C. 5.3.1.1], leucine amino peptidase (LAP) [3.4.11.1], phosphoglucomutase (PGM) [5.4.2.2], and phosphoglucoisomerase (PGI) [5.3.1.9], since these were the only ones which could be scored with confidence, although many other enzyme systems showed activity.

TPI, PGM, and LAP were resolved on a gel using a lithium-citrate buffer (Ridgway et al., 1970) and PGI was resolved using the citrate-morpholine buffer of Clayton and Tretiak (1972), modified according to Nickrent (1984, Ph.D. dissertation, Miami University, Oxford, Ohio).

In addition to the enzymes exhibiting bands of adequate intensity and resolution, the following enzyme systems were examined: acid phosphatase, aconitase, adenylate kinase, alcohol dehydrogenase, aldolase, cytochrome oxidase, diaphorase, esterase, fructose 1,6-diphosphatase, glyceraldehyde-3-phosphate dehydrogenase, glycerate-2-dehydrogenase, glucose-6-phosphate dehydrogenase, glutamate dehydrogenase, glutamate oxaloacetate transaminase, glutathione reductase, hexokinase, isocitrate dehydrogenase, maleate dehydrogenase, malic enzyme, menadione reductase, peroxidase, 6-phosphogluconate dehydrogenase, poly-

phenol oxidase, and shikimic acid dehydrogenase. Although most of these enzymes showed activity in extracts from some plants, because of lack of sufficient intensity, resolution, or repeatability, none of them was found suitable for analysis. Unusually high levels of phenolic compounds are almost certainly responsible for the loss of enzyme activity in these species of *Aster*.

Because of the difficulty in obtaining interpretable banding patterns for many of the enzymes listed, a number of compounds were added to the grinding buffer to try to improve resolution and intensity. Substances added to the grinding buffer included: .5 M ascorbate, bovine serum albumin, casein, cysteine, diethyldithiocarbamic acid, diethyldithiothrietol, dimethylsulfoxide, ethylenediamine tetraacetic acid, ethanol, glycerol, higher concentrations of metabisulfite, nicotinamide adenine dinucleotide, nicotinamide adenine dinucleotide phosphate, phenylmethylsulfonyl fluoride, polyethylene glycol, pyridoxine-5′-phosphate, sucrose, thiourea, and Tween 80 (a detergent). These additions were done using various 2^{p-k} fractional factorial designs (Box and Draper, 1987), which would have indicated if any of these substances was useful either alone or in combination. However, none of them improved resolution or intensity over that achieved with the grinding buffer recipe listed above.

Many other gel and electrode buffer combinations were used in an attempt to obtain repeatable and interpretable enzyme bands and to improve band resolution and intensity. None resolved the isozymes any better than those produced on gels prepared with the lithium-citrate or citrate-morpholine buffers. Similarly, use of tissue from the very youngest leaves did not significantly improve the results.

Enzyme staining protocols were derived from Soltis et al. (1983). For enzyme systems with two or more loci present on a gel, the fastest moving (most anodal) locus was designated as number one. Alleles at a locus were designated by capital letters, with A being the most anodally migrating allele. Allelic frequencies were estimated for the polyploids based on visual inspection of the intensity of band staining (Jorgensen, 1986; Nielsen, 1980) and analyzed phenetically using the program BIOSYS-1 (Swofford and Selander, 1981).

Because determination of gene frequencies for polyploids is not as straightforward as for diploids, a brief explanation on how gene frequencies were determined is necessary. Consider a banding

pattern for TPI-1 (a dimer) for a specimen of the tetraploid, *A. chlorolepis*, where the intensities of evenly spaced bands from top (anode) to bottom (cathode) are approximately 1:2:4:4:4. Based on banding patterns from diploid homozygotes, the faintest band occurs at the position of allele A, the middle band is located at the position of allele B, and the lowest band at allele C. The simplest explanation for these band intensities at these positions is that there is one copy of allele A, one of allele B, and two of allele C. In this case, the actual band intensities would be 1:2:5:4:4, with the AC dimer occurring at the same position as the BB dimer.

Because no more than 4 different alleles were found for the dimeric enzymes TPI-1, TPI-2, and PGI-2, estimation of gene dosages were, even for the most complex patterns, not much more difficult to interpret than that described above. In every case, the simplest explanation that would account for the observed banding pattern was accepted as correct. Fortunately, the genetic distance measures of Nei (1972, 1978) and Rogers (1972) are not overly sensitive to small differences in gene frequencies, so that minor errors in estimating gene dosages do not change the resulting phenograms. In addition, the most variable enzyme, PGM-2, is a monomer, so that the determination of gene dosages for this enzyme is not confused by overlapping bands that represent different allelic combinations.

In order to determine gene frequencies for a species, it was assumed that each plant carries two copies of every gene for every copy of the diploid genome it possessed. Thus, for example, an octoploid plant must carry 8 copies of the PGM-2 gene, and the enzyme banding intensities must reflect this. The total number of PGM-2 genes in the sample of 44 *A. macrophyllus* plants that must be accounted for is then 8 times the number of individuals, that is, $8 \times 44 = 352$. Thus since allele G of PGM-2, for example, was found 4 times out of 352, it has a gene frequency of $4/352 \times 100\% = 1\%$ (with rounding). Gene frequencies were computed similarly for other enzymes and other species. Since Nei's and Rogers's genetic distance measures depend only on gene frequencies, and not on ploidy level, these quantities were computed by the computer program BIOSYS-1 and used in the phenetic analyses.

Five assumptions are involved in determining gene frequencies as described above. The first two are that there are no null alleles

in any of the taxa and no inactive loci in the polyploids. Null alleles cannot definitively be detected without crossing experiments, and these could not be conducted because Biotian asters will not bloom under our greenhouse conditions. The third and fourth assumptions are that all forms of an enzyme have the same enzyme activity and that all duplicate loci in the polyploids are homologous, thus allowing dosages to be estimated from banding intensity, ploidy level, and knowledge of enzyme quaternary structure. The fifth is that there have been no gene duplications in the polyploids above those resulting from polyploidy itself.

RESULTS AND DISCUSSION

Four enzyme systems representing seven presumed loci were analyzed: TPI-1, TPI-2, LAP, PGI-1, PGI-2, PGM-1 and PGM-2. The estimated allelic frequencies for alleles at these loci are shown in Table 1. Three alleles were found for TPI-1, TPI-2, and LAP, seven were found for PGM-2, and four were found for PGI-2. PGI-1 and PGM-1 were found to be monomorphic across all species in the section.

Two isozymes of TPI, PGM, and PGI were detected in diploid members of *Aster* section *Biotia*. This number is usually found for these enzymes (e.g., Gottlieb, 1982; Gottlieb et al., 1985). Only one isozyme of LAP was found. Previous workers reported either one or two loci for LAP (Bayer and Crawford, 1986; Crawford and Bayer, 1981; Crawford and Smith, 1984; Gottlieb et al., 1985; Heywood and Levin, 1984; Warwick and Gottlieb, 1985). Thus, none of the diploid members of *Aster* section *Biotia* appear to have duplicated loci, which, if found, might have been of help in assessing the ancestry of the polyploid taxa. Because of the method of computing gene frequencies in polyploids, it was not possible to determine if they possessed any duplicate loci not possessed by the diploids.

Since PGI, PGM, and TPI are known to be found both in the plastids and the cytosol in other plant taxa (Gottlieb, 1982), these are assumed to be their subcellular locations in members of *Aster* section *Biotia*. Since LAP is not known from plastids, this enzyme is probably cytosolic in these *Aster* species also.

When all species in the section are considered, a total of four loci was found to be fixed for a single allele. TPI-1 is fixed at allele C in *Aster mirabilis* and *A. jonesiae*. This finding is evidence

Table 1. Allelic frequencies for the seven species in *Aster* section *Biotia*. Species names are abbreviated using the first three letters of the specific epithet.

	Species						
Locus	div	fur	mir	chl	jon	sch	mac
TPI-1							
(N)	70	18	19	12	4	16	44
A	.057	.000	.000	.479	.000	.083	.108
B	.121	.639	.000	.104	.000	.250	.412
C	.822	.361	1.000	.417	1.000	.667	.480
TPI-2							
(N)	70	18	19	12	4	16	44
A	.250	.028	.237	.333	.625	.365	.324
B	.700	.972	.684	.667	.375	.635	.625
C	.050	.000	.079	.000	.000	.000	.051
LAP							
(N)	70	18	19	12	4	16	44
A	.100	.000	.000	.000	.000	.000	.023
B	.850	1.000	.684	.958	.500	.781	.920
C	.050	.000	.316	.042	.500	.219	.057
PGM-2							
(N)	70	18	19	12	4	16	44
A	.014	1.000	.000	.104	.000	.000	.088
B	.158	.000	.105	.396	.500	.281	.415
C	.021	.000	.000	.000	.000	.000	.006
D	.800	.000	.605	.500	.500	.719	.441
E	.000	.000	.290	.000	.000	.000	.028
F	.000	.000	.000	.000	.000	.000	.011
G	.007	.000	.000	.000	.000	.000	.011
PGI-2							
(N)	70	18	19	12	4	16	44
A	.164	.056	.000	.000	.000	.000	.074
B	.529	.944	.026	.500	.500	.281	.418
C	.300	.000	.974	.500	.500	.625	.494
D	.007	.000	.000	.000	.000	.094	.014

for a close relationship between the diploid, *A. mirabilis,* and the hexaploid, *A. jonesiae.* LAP is fixed at allele B and PGM-2 is fixed at allele A in *A. furcatus.* Since the B allele for LAP is the most common allele in all the species, no inference can be based on its fixation in *A. furcatus.* Allele A for PGM-2, however, is rare or absent in the other members of the section, which helps account for the relatively distant relationship of *A. furcatus* to the

Table 2. Mean unbiased heterozygosity and mean number of alleles per locus for species in *Aster* section *Biotia*. Results from the loci that are monomorphic across all species (PGI-1 and PGM-1) are included.

Species	Mean Unbiased Heterozygosity per Locus	Mean Number of Alleles per Locus
A. divaricatus	.281	2.9
A. furcatus	.091	1.4
A. mirabilis	.219	1.9
A. chlorolepis	.327	2.0
A. jonesiae	.321	1.6
A. schreberi	.327	2.0
A. macrophyllus	.353	3.1

rest of the species in the section. *Aster divaricatus, A. chlorolepis, A. schreberi* and *A. macrophyllus* were polymorphic for all loci examined.

The only allele unique to a species is PGM-2 allele F in *Aster macrophyllus,* and it is found at a very low frequency. It may represent an allele that was obtained by the octoploid species from some *Aster* species outside of the section, an allele that arose *de novo* in the species, or an allele present in the other species but that escaped detection owing to sampling variability.

Based on the values for mean unbiased heterozygosity per locus (Table 2), six of the Biotian asters are in the normal range of variability as compared to most other angiosperms, which have heterozygosity values in the range .20 to .35. The low heterozygosity value (.091) for *Aster furcatus* may be a consequence of widely separated populations and a relatively higher degree of self-compatibility (Les, pers. comm.).

Values for Nei's unbiased genetic identity and Rogers's genetic similarity were computed from gene frequencies and are displayed in Table 3. Phenograms constructed by the unweighted pair group method with arithmetic averages (Sneath and Sokal, 1973) using Nei's unbiased genetic identity measure (Figure 2) or Rogers's genetic similarity measure (Figure 3) show the phenetic relationships between the seven species in the group. In both figures, *Aster chlorolepis* and *A. macrophyllus* are grouped first, and then *A. divaricatus* and *A. schreberi* are joined. These two clusters are then united together to form the set consisting of the eastern North American deciduous forest species of *Aster* section *Biotia.*

Table 3. Matrix of genetic similarity and identity coefficients for species in *Aster* section *Biotia*. Above diagonal: Rogers's genetic similarity. Below diagonal: Nei's unbiased genetic identity.

Species	1	2	3	4	5	6	7
1 *A. divaricatus*	*****	.570	.746	.789	.715	.837	.809
2 *A. furcatus*	.669	*****	.397	.578	.385	.509	.625
3 *A. mirabilis*	.854	.448	*****	.663	.726	.785	.688
4 *A. chlorolepis*	.916	.700	.804	*****	.721	.813	.889
5 *A. jonesiae*	.895	.495	.894	.851	*****	.750	.727
6 *A. schreberi*	.959	.603	.936	.948	.947	*****	.849
7 *A. macrophyllus*	.924	.740	.825	.974	.868	.969	*****

Next, *Aster jonesiae* and *A. mirabilis* are joined to the four-species group, which is the only topological difference between the two figures. In Figure 2, *A. jonesiae* and *A. mirabilis* first are joined in a two-taxon cluster, which is united to the cluster of the four previous species. By contrast, in Figure 3, first *A. jonesiae* is joined into a five-species group, and a very short distance later, *A. mirabilis* is united with the previous five species. Although Figures 2 and 3 differ slightly in the clustering of *A. jonesiae* and *A. mirabilis,* both emphasize the close relationship between these two Piedmont species.

Finally, in both Figures 2 and 3, *Aster furcatus* is joined to the remaining six species at a relatively low level of genetic similarity.

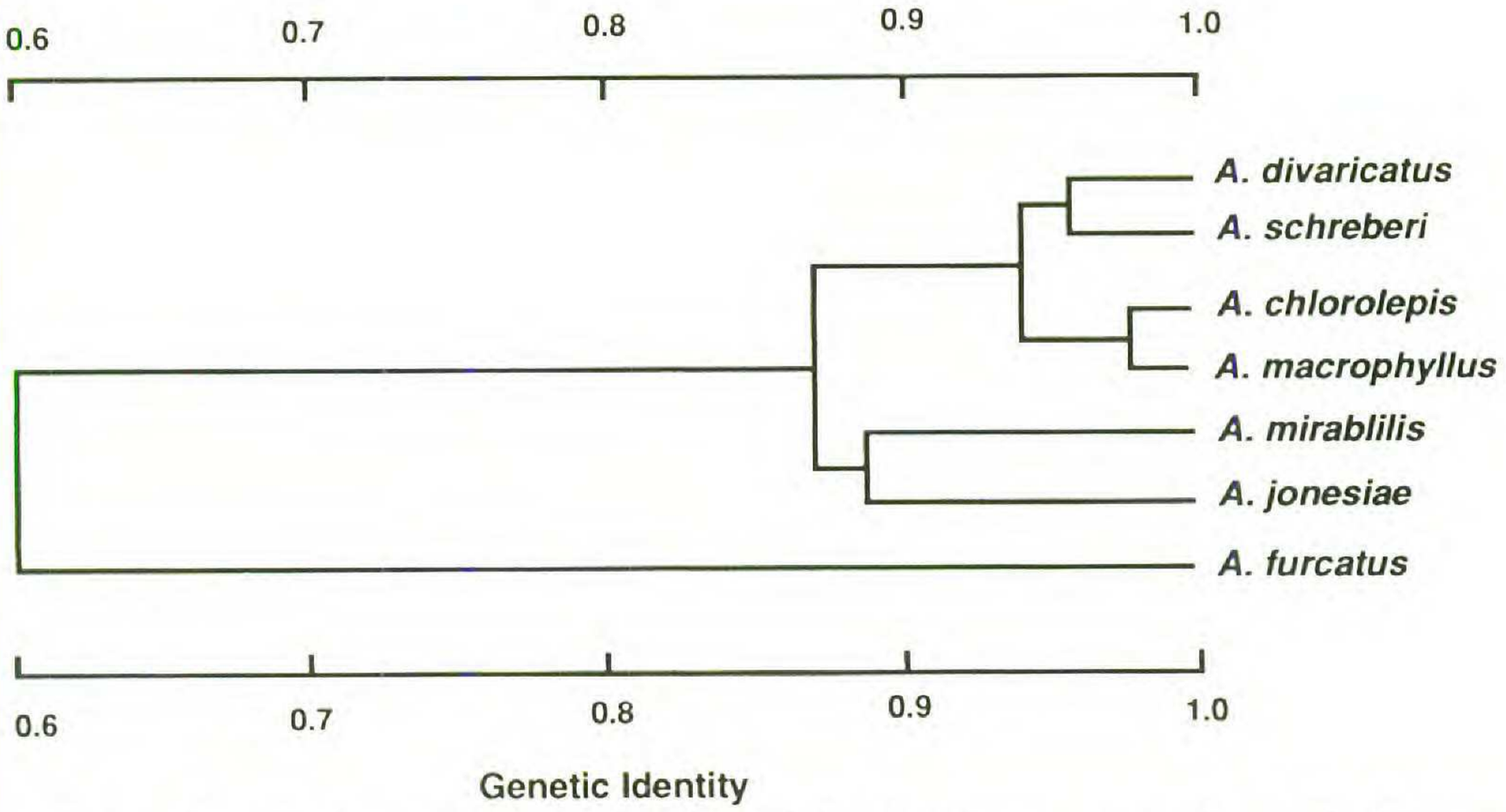

Figure 2. Cluster analysis using unweighted pair group method. Coefficient used: Nei's (1978) unbiased genetic identity. Abbreviations are as in Table 1.

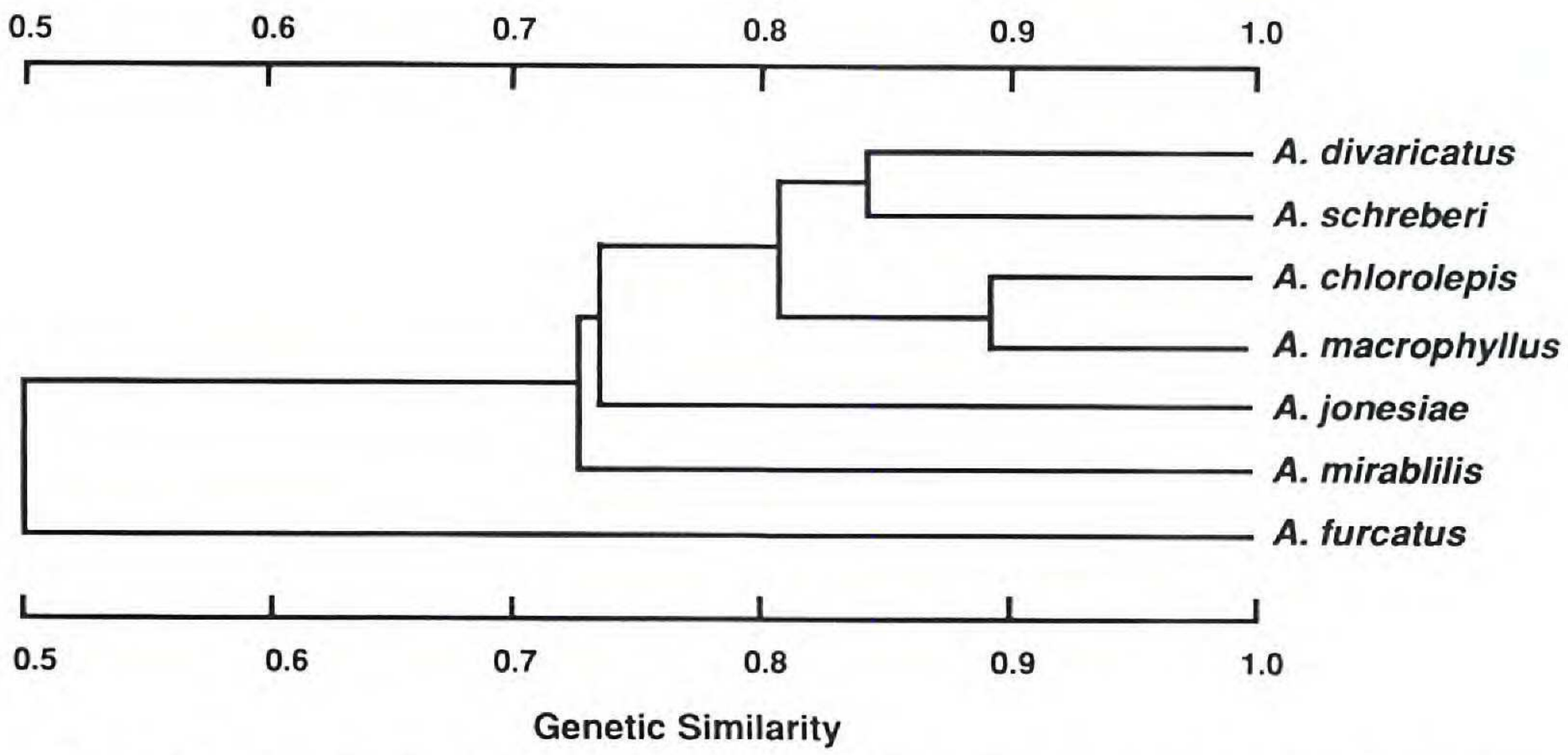

Figure 3. Cluster analysis using unweighted pair group method. Coefficient used: Rogers's (1972) genetic similarity. Abbreviations are as in Table 1.

Phenograms constructed using either weighted pair group clustering with arithmetic averages or complete linkage clustering (Sneath and Sokal, 1973) show the same topologies as those in Figures 2 and 3.

The relationships shown in Figures 2 and 3 are precisely those one would predict from phytogeographic information. *Aster divaricatus, A. chlorolepis, A. schreberi* and *A. macrophyllus* are native to eastern and central North America north of the mountains of Alabama, Georgia, and South Carolina. *Aster mirabilis* and *A. jonesiae* are restricted in range to the Piedmont of Alabama, Georgia, South Carolina, and North Carolina, and the single species, *A. furcatus,* is a midwestern endemic.

Previous isozyme studies by other workers have shown that conspecific populations usually have genetic identities of .90 and above (Crawford, 1983). When different species have genetic identities this high, they are often assumed to have diverged relatively recently. If species limits in *Aster* section *Biotia* were determined solely on the basis of the .90 criterion for conspecificity, *A. divaricatus, A. chlorolepis, A. schreberi,* and *A. macrophyllus* would be considered to be members of the same species (Figure 2). Because the morphologies and chromosome numbers of *A. divaricatus, A. chlorolepis, A. schreberi,* and *A. macrophyllus* differ, however, these taxa have been maintained as distinct (Lamboy, 1990, op. cit.). This interpretation is not without precedent. For example, Nickrent (1986) also found several pairs of *Arceuthobi-*

um species with genetic identities greater than .90, but he did not regard them as conspecific. The high values for the genetic identities of the four Biotian asters indicate, however, that these taxa may have diverged from an ancestral taxon relatively recently, perhaps since the last glaciation.

Lamboy (1991, at review) has argued that *Aster chlorolepis* is an autotetraploid derivative of *A. divaricatus.* The isozyme data support this conclusion in two ways. First, there is no evidence of the fixed heterozygosity in *A. chlorolepis* that would be expected in an allotetraploid. In addition, the species maintains at least 3 alleles at two loci, TPI-1, and PGM-2, and it shows both homozygous and heterozygous banding patterns. Finally, it has a greater heterozygosity than its putative progenitor (Table 3), which is what would be expected in an autopolyploid (Soltis and Rieseberg, 1986).

Lamboy and Jones (1988) hypothesized that *Aster jonesiae* arose by retrogressive polyploidy from a hybrid of *A. mirabilis* and *A. macrophyllus.* All plants of *A. jonesiae* examined display the predicted fixed heterozygosity of such an origin. *Aster jonesiae* and *A. mirabilis* are also the only two species fixed for allele C at TPI-1. There is, thus, some evidence supporting the hypothesis of origin by retrogressive polyploidy, but confirmation is desirable.

The suggestion that *Aster schreberi* arose by retrogressive polyploidy (Lamboy and Jones, 1988) from a hybrid of *A. divaricatus* and *A. macrophyllus* also is supported by the isozyme data. Although there is no evidence for the fixed heterozygosity expected in a taxon of allopolyploid origin (Soltis and Rieseberg, 1986), if *A. schreberi* or its hybrid progenitor originated independently several times, fixed heterozygosity might not be evident in the observed genotypes. Since the ranges of the putative ancestors, *A. macrophyllus* and *A. divaricatus,* overlap through much of New England, New York, Pennsylvania, Ohio, and Virginia (Lamboy, 1990, op. cit.), multiple origins of *A. schreberi* or its hybrid progenitor would not be unexpected. Furthermore, *A. schreberi* has its greatest genetic identity with *A. macrophyllus,* and its second greatest with *A. divaricatus,* and every allele found in *A. schreberi* is also found in both *A. divaricatus* and *A. macrophyllus.* Thus, the isozyme data provide substantial evidence supporting the origin of *A. schreberi* by retrogressive polyploidy.

Finally, the isozyme data are inconclusive concerning the derivation of the octoploid, *Aster macrophyllus.* Among the taxa

examined, this species has the greatest genetic identity with *A. chlorolepis* and the second greatest with *A. schreberi*. One allele, E at PGM-2, present in low frequency, *A. macrophyllus* shares only with *A. mirabilis*. These inconsistent results leave the relationship and origins of *A. macrophyllus* obscure.

This study represents the first published information on isozymes in the genus *Aster*. It must be emphasized that because of the limited number of loci and small number of plants of some species, the conclusions reached cannot be considered definitive. Nevertheless, the electrophoretic data do corroborate some of the results obtained from chromosome numbers, morphological measurements, and phytogeographic information. Analysis of the gene frequencies of isozyme alleles in *Aster* species still holds great potential for answering taxonomic and phylogenetic questions in the genus, since it is the best method for obtaining genotype information on large numbers of individuals at a reasonable cost. If this potential is to be realized, however, the problem of loss of enzyme activity due to unusually high concentrations of phenolic compounds in *Aster* must be overcome.

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APPENDIX

Vouchers for Living Specimens Used in the Isozyme Analyses

All numbers are Lamboy collections unless otherwise noted. Numbers in parentheses indicate number of plants analyzed that belonged to the population from which the voucher plant was taken. All specimens are deposited at ILL.

Aster chlorolepis. **North Carolina:** Caldwell Co., *303, 305, 306,* Swain Co., *313, 315, 316, 317,* Yancey Co., *310, 430*; **Tennessee:** Carter Co., *426*; **Virginia:** Grayson Co., *415, 417.*

Aster divaricatus. **Alabama:** Marshall Co., *261, 262*; **Connecticut:** Fairfield Co., *170,* Litchfield Co., *246,* New Haven Co., *171, 176*; **Georgia:** Rabun Co., *319, 439*; **Kentucky:** Carter Co., *395, 396, 398*; **Massachusetts:** Berkshire Co., *181, 248, 249,* Bristol Co., *189,* Franklin Co., *254,* Norfolk Co., *190,* Worcester Co., *192*; **New Hampshire:** Cheshire Co., *201,* Rochester Co., *Jones 4338*; **New Jersey:** Warren Co., *161, 162*; **New York:** Cattaraugus Co., *239, 241,* Suffolk Co., *164, 167,* Ulster Co., *243*; **North Carolina:** Caldwell Co., *300, 302,* Henderson Co., *432, 433,* Jackson Co., *434, 435,* Macon Co., *438,* Swain Co., *322, Jones 3440,* Wilkes Co., *413,* Yancey Co., *307*; **Ohio:** Hocking Co., *380, 386, 389, 390, 393,* Jefferson Co., *74,* Vinton Co., *382,* **Pennsylvania:** Allegheny Co., *80,* Clarion Co., *126, 127, 129, 134, 138,* McKean Co., *222,*

Westmoreland Co., *43, 47*; **Tennessee:** Carter Co., *424, 425*, **Vermont:** Windsor Co., *216*; **Virginia:** Giles Co., *411*, Grayson Co., *414, 422*, Page Co., *408*, Smyth Co., *418, 419, 420, 423*; **West Virginia:** Grant Co., *404*, Hardy Co., *406*, Pendelton Co., *402, 403*, Randolph Co., *399*.

Aster furcatus. **Illinois:** LaSalle Co., *4, 5, 6* (3), *443, 447, 448, 449, 450, Jones 6228* (2); **Indiana:** Warren Co., *444, 445, Jones 6524* (2); **Iowa:** Muscatine Co., *Watson s.n.* (2); **Wisconsin:** Fond du Lac Co., *224*.

Aster jonesiae. **Georgia:** Harris Co., *264, 265*, Morgan Co., *273, 274*.

Aster macrophyllus. **Connecticut:** Litchfield Co., *178*; **Massachusetts:** Franklin Co., *194*, Norfolk Co., *191*, Worcester Co., *193*; **Michigan:** Benzie Co., *156*, Delta Co., voucher lost, Grand Traverse Co., *Jones 5675* (2), Mason Co., *143, 154, 160, 442*; **New Hampshire:** Coos Co., *211*, Grafton Co., *213*; **New York:** Cattaraugus Co., *240, 242*, Essex Co., *220*; **North Carolina:** Henderson Co., *431*, Yancey Co., *309, 429*; **Ohio:** Hocking Co., *384, 387, 391, 392, 395*; **Pennsylvania:** Allegheny Co., *64*, Clarion Co., *119, 134*, Clearfield Co., *86*, Venango Co., *90, 94*; **Vermont:** Windsor Co., *217*; **Virginia:** Giles Co., *410, 412*, Page Co., *407, 409*; **West Virginia:** Greenbrier Co., *Jones 6036*, Pendleton Co., *401*, Randolph Co., *400*; **Wisconsin:** Fond du Lac Co., *225, 228*, Sauk Co., *17, 34*, Washington Co., *230*.

Aster mirabilis. **South Carolina:** Chester Co., *280, 281, 282, 283, 284, 285, 286, 288*, Lancaster Co., *289, 290, 293, 295, 297, 298, 440*, Richland Co., *Jones 6185* (4).

Aster schreberi. **Connecticut:** Hartford Co., *183*; **Illinois:** Peoria Co., *7* (2), *446, Ebinger 23678, Jones 6202*; **Kentucky:** Carter Co., *397*; **Massachusetts:** Berkshire Co., *247*; **New York:** Ulster Co., *245*; **Ohio:** Hocking Co., *381, 383, 388*, Morrow Co., *378, 379, 441*; **Vermont:** Bennington Co., *196*.